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Immunohistochemistry on free-floating cryosections

Forked from Immunochemistry on paraffin sections

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In 3 collections

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We use this protocol and it's working

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Abstract

Immunochemistry protocol on rodent brain cryosections

Materials

Reagents :

- TBS 10X : Tris base 121.1g + NaCl 90g in 1L H2O.pH 7.4.
- TBS 1X-Triton 0,5%
- Xilen
- Ethanol : 100%, 95%, 70%
- Unmasking buffer epitopes : Citrate solution 10mM pH6.0
- Blocking Buffer : TBS 1X + 5% NGS
- 1st Ab : Diluted in1X PBS +2%NGS
- 2nd Ab : Diluted in1X PBS +2%NGS
- Endogenous peroxidase blocking solution : TBS 1x + 3% H2O2(30%) + 10% methanol

1. Section selection

1 Collect the cryosections needed for a caudo-rostral representation of each brain region (every fourth section or every sixth section dependeing on the section thinknes, brain region and animal species) into 24-well-plate (3-4 sections per well).

1.1 Wash 3×5 min in TBS 1X. Put 500ul per well and aspirate the liquid with an air-pump equipment.

2. Blocking endogenous peroxidase

2 Incubate sections in endogenous peroxidase blocking solution: TBS 1x + 3% H2O2 + 10% methanol for 10 min (500uL/well)

2.1 Wash 3×5 min in TBS 1X.

3. Blocking

3 Incubate sections in blocking in TBS 1X + 5% NGS or NDS (500uL/well) 1h at RT

4. 1ary antibody

4 Incubate sections inTBS 1X + 2% NGS or NDS + primary Ab 24/72h (it depends of the Ab) at 4ºC (cold room).

4.1 Wash 3×5min in TBS 1X.

5. 2ary antibody

5 Incubate sections in 2% NGS or NDS + Secondary Ab 1h at RT.

5.1 At this step it is important to prepare ABC solution and let it, at least, 30 min on the shaker

5.2 Wash 3×5min in TBS 1X.

6. ABC incubation

6 Incubate 1 hour at RT with ABC solution (Ultra-Sensitive or Standard ABC Peroxidase Standard Staining Kit).

6.1 Wash 3×5min in TBS 1X.

7. Developing

7 In aluminium foil: DAB Standard Kit (1 drop of reagent B in 1 mL of reagent A, gives rise to brown staining), or Vector SG (3 drops Chromogen + 3 drops Hydrogen Peroxide in 5mL PBS, gives rise to blue staining).

8 Put 500 uL on each well and put a cardboard box on it to keep darkness for a time ranging from 3-15 minutes depending on the antibody used.

8.1 Remove with an air-pump equipment and clean the material with bleach.

8.2 Wash 3×5min in TBS 1X.

8. Mount sections

9 Mount sections into slides and let it dry overnight.

9. Dehydratation

10 Incubate slides in consecutive ethanol solutions (1 min in ethanol 70%-1 min in ethanol 95%-1 min in ethanol 100%).

11 Incubate slides in 2×5 min in Xylene.

10. Mount coverslips

12 Put a line of mounting medium (DPX) by slide. Put the coverslip (washed with ethanol previously) on the slide. Remove bubbles.

13 Let dry the slides in the hood overnight.